

## CLAIMS

1. A method of producing a *thy*<sup>-</sup> strain of *Vibrio cholerae* comprising the  
5 step of site-directed mutagenesis in the *V. cholerae* chromosome for the deletion and/or  
insertion of gene nucleotides at the locus of the *thy* A gene having essentially the  
nucleotide sequence SEQ ID NO: 1 of FIG. 1.
2. A *Vibrio cholerae thy*<sup>-</sup> strain which is a  $\Delta$  *thy* A strain lacking the  
functionality of the *thy* A gene.
- 10 3. A  $\Delta$  *thy* A strain of *Vibrio cholerae* according to claim 2 comprising one  
or several episomal autonomously replicating DNA elements having a functional *thy* A  
gene that enables the strain to grow in the absence of thymine in the growth medium.
4. A  $\Delta$  *thy* A strain of *Vibrio cholerae* according to claim 3, wherein the  
episomal autonomously replicating DNA element is a plasmid.
- 15 5. A  $\Delta$  *thy* A strain of *Vibrio cholerae* according to claim 3 or 4 comprising  
a foreign *thy* A gene.
6. A  $\Delta$  *thy* A strain of *Vibrio cholerae* according to claim 5, wherein the  
foreign *thy* A gene is an *E. coli* gene.
7. A  $\Delta$  *thy* A strain of *Vibrio cholerae* according to any one of claims 3 to  
20 6, wherein the one or several episomal autonomously replicating DNA elements also  
comprise a structural gene encoding a homologous or heterologous protein.
8. A  $\Delta$  *thy* A strain of *Vibrio cholerae* according to claim 7, wherein the  
encoded protein is selected from heat labile enterotoxin B-subunit of *Escherichia coli*  
(LTB) and *Schistosoma japonicum* glutathione S-transferase 26 kD protein (GST 26  
25 kD).
9. A nucleotide sequence of a *thy* A gene of *Vibrio cholerae* having  
essentially the nucleotide sequence SEQ ID NO: 1 of FIG. 1.
10. A nucleotide sequence of a 5'-flanking region of a structural *thy* A  
gene of *Vibrio cholerae* having essentially the nucleotide sequence SEQ ID NO: 2 of  
30 FIG. 2.
11. A nucleotide sequence of a 3'-flanking region of a structural *thy* A  
gene of *Vibrio cholerae* having essentially the nucleotide sequence SEQ ID NO: 3 of  
FIG. 3.

12. A protein encoded by a nucleotide sequence of a *thy A* gene of *Vibrio cholerae* according to claim 9.

13. A protein according to claim 12, wherein the protein has the amino-acid sequence SEQ ID NO: 4 of FIG. 4.

5            14. A protein encoded by a nucleotide sequence of a 5'-flanking region of  
a structural *thyA* gene of *Vibrio cholerae* according to claim 10.

15. A protein according to claim 14, wherein the protein has the amino-acid sequence SEQ ID NO: 5 of FIG. 5.

16. A vaccine comprising as an immunising component a *Vibrio cholerae*  
 10  $\Delta thy$  A strain according to any one of the claims 2 - 8 or a *thy* A<sup>-</sup> strain of *Vibrio*  
*cholerae* produced by the method of claim 1.